Nucleosome positioning as a critical determinant for the DNA cleavage sites of mammalian DNA topoisomerase II in reconstituted Simian virus 40 chromatin

Giovanni Capranico+, Christine Jaxel§, Michel Roberge$^{\phi}$, Kurt W.Kohn and Yves Pommier*
Laboratory of Molecular Pharmacology, Division of Cancer Treatment, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892, USA

Received March 21, 1990; Revised and Accepted July 5, 1990

ABSTRACT

We have assessed the ability of nucleosomes to influence the formation of mammalian topoisomerase II-DNA complexes by mapping the sites of cleavage induced by four unrelated topoisomerase II inhibitors in naked versus nucleosome-reconstituted SV40 DNA. DNA fragments were reconstituted with histone octamers from HeLa cells by the histone exchange method. Nucleosome positions were determined by comparing micrococcal nuclease cleavage patterns of nucleosome-reconstituted and naked DNA. Three types of DNA regions were defined: 1) regions with fixed nucleosome positioning; 2) regions lacking regular nucleosome phasing; and 3) a region around the replication origin (from position 5100 to 600) with no detectable nucleosomes. Topoisomerase II cleavage sites were suppressed in nucleosomes and persisted or were enhanced in linker DNA and in the nucleosome-free region around the replication origin. Incubation of reconstituted chromatin with topoisomerase II protected nucleosome-free regions from micrococcal nuclease cleavage without changing the overall micrococcal nuclease cleavage pattern. Thus, the present results indicate that topoisomerase II binds preferentially to nucleosome-free DNA and that the presence of nucleosomes at preferred DNA sequences influences drug-induced DNA breaks by topoisomerase II inhibitors.

INTRODUCTION

The structural unit of eukaryotic chromatin is the nucleosome core particle, which consists of about 146 base pairs (bp) of DNA wrapped in 1.8 turns around an octamer of histone proteins (1–3). Histones and DNA can readily associate to form the nucleosome subunits of chromatin and several experimental systems have been designed to reconstruct and analyze the cellular mechanisms of chromatin assembly (4,5). In addition to the simple repeating subunit, the nucleosome particle, chromatin is folded into solenoid structures which are attached to the nuclear scaffold at specific DNA sequences (6,7). The findings that DNA topoisomerase II (8,9) is positioned at the base of loop domains in the chromosome scaffold (10–12) and in the nuclear matrix of interphase cells (13), suggest that topoisomerase II plays a crucial role in maintaining the chromatin structure of living cells. However, the precise mechanisms by which the enzyme binds to DNA and may control *in vivo* DNA topology and chromatin structure are not completely understood (8,9).

The sites of topoisomerase II catalytic activity correspond to enzyme-induced and -linked DNA breaks (8,9). The DNA cleavage activity of mammalian DNA topoisomerase II can be enhanced at specific DNA sequences by several classes of antitumor drugs, resulting in protein-linked DNA breaks in cells as well as in *in vitro* systems (14–16). Consequently, topoisomerase II cleavage sites were mapped in chromatin and found to be a subset of those produced by the purified enzyme in naked DNA (17–21). This can be due to enzyme partitioning in the nucleus or to reduced accessibility of the DNA to topoisomerase II.

In the present study, we have assessed the ability of nucleosomes to influence the position of topoisomerase II-DNA complexes. Simian virus 40 (SV40) DNA, which forms minichromosomes *in vivo* was used as a substrate for nucleosome reconstitution experiments, and four chemically unrelated drugs, teniposide (VM-26), amsacrine (m-AMSA), 5-iminodaunorubicin and 2-methyl-9-hydroxyellipticinium, that are known to induced enzyme-mediated DNA cleavage at specific sequences, were used to stabilize topoisomerase II-DNA complexes (14–16).

MATERIALS AND METHODS

DNA fragments and labeling

Two fragments encompassing most of the SV40 genome were used. The Ban I/Hpa II DNA fragment was uniquely labeled at the Ban I restriction site (genomic position 298 bp) and was 5188 bp long (second cut with Hpa II at position 347). The Acc I/Eco RI DNA fragment was labeled at the Acc I restriction site

* To whom correspondence should be addressed

Present addresses: +Istituto Nazionale per lo Studio e la Cura dei Tumori, via G. Venzian 1, 20133 Milan, Italy, § Laboratoire d'Enzymologie des Acides Nucleiques, Universite Pierre et Marie Curie, 96 Blvd. Raspail, 75006 Paris, France and $^{\phi}$ESREC, chemin des Boveresses 155, CH 1066 Epalinges, Switzerland

(genomic position 1628 bp) and then digested with Eco RI, resulting in a 5089 bp fragment. In some experiments, the Ban I and the Acc I fragments were digested with Taq I and Bam HI, respectively. Prior to end labeling the 5'-DNA termini were dephosphorylated with calf alkaline phosphatase in order to improve the end labeling efficiency with [γ-^{32}P]ATP and T4 polynucleotide kinase.

Nucleosome reconstitution of SV40 DNA fragments

DNA fragments were reconstituted in the nucleosomal state as described by Drew and Travers (5). Nucleosome core particles purified from HeLa cells (22−24) were kindly provided by Dr. Brian Imai (Department of Biological Chemistry, University of California, Davis, CA 95616). 5'-end labeled SV40 DNA fragments were mixed with a large excess of nucleosome core particles (1 mg/ml) in 20 μL (core particles/DNA ratio = 20) containing 0.7 M NaCl, 20 mM Tris-HCl, 0.2 mM EDTA, pH 8. After 20 min at 37°C, reaction mixtures were diluted to 200 μL by the stepwise addition of 5 mM Tris-HCl (pH 8), 0.1% Nonidet-P40 (5 μL each 10 min) at 20°C. A small part of the reconstituted mixture was then loaded into a 0.7% agarose gel in 0.5× TBE (1× TBE: 89 mM Tris, 89 mM Boric acid, 2 mM EDTA, pH 8) to check the efficiency of reconstitution (5). In order to determine nucleosome positioning, equal amounts of DNA from both naked and nucleosome-reconstituted DNA samples were adjusted to 2 mM $CaCl_2$ and micrococcal nuclease was allowed to cut for 0.5 to 8 min at 37°C. Reactions were stopped by adding SDS and EDTA (final concentrations, 1% and 10 mM, respectively). Nucleosome-reconstituted DNA samples were more sensitive to micrococcal nuclease digestion than naked DNA, probably due to residual enzyme molecules used during the preparation of mononucleosomes from HeLa cells. The relative concentrations of the enzyme were adjusted so that both free and nucleosome-reconstituted DNA samples were cut to similar extents. Micrococcal nuclease digestion patterns were analyzed by means of 1.2% agarose gel electrophoresis.

Topoisomerase II cleavage of SV40 DNA

DNA topoisomerase II was purified from murine L1210 cells as already described (25). The purified enzyme yielded a single 170 kDa band after silver staining of SDS-polyacrylamide gels. Topoisomerase II reactions were essentially as described (21). Naked and nucleosome-reconstituted DNA fragments were reacted with 60 ng of topoiso-merase II with or without drugs (10 μM VM26, 10 μM *m*-AMSA, 2 μM 5-iminodaunorubicin, or 1 μM 2-methyl-9-hydroxyellipticinium) in 0.01 M Tris-HCl, pH 7.5, 0.05 M KCl, 5 mM $MgCl_2$, 0.1 mM EDTA and 1 mM ATP 20 min at 37°C. Topoisomerase II was added to each 20 μL reaction volume in 3 μL of storage buffer [30% (v/v) glycerol, 0.35 M NaCl, 5 mM $MgCl_2$, 1 mM EGTA, 1 mM KH_2PO_4, 0.2 mM dithiothreitol and 0.1 mM phenylmethanesulfonyl fluoride, pH 6.4]. Reactions were stopped by adding SDS, EDTA and proteinase K (final concentrations 1%, 10 mM and 250 μg/ml, respectively) and samples incubated for 1 h at 42°C. Samples were then loaded into 1.2% agarose gels in TBE buffer. Samples to be analyzed for DNA sequencing were ethanol-precipitated after proteinase K treatments and resuspended in 2.5 μL loading buffer (80% formamide, 10 mM NaOH, 1 mM EDTA, 0.1% xylene cyanol and 0.1% bromophenol blue). Samples were heated at 90°C before loading into DNA sequencing 8% polyacrylamide (29:1, acrylamide:bis) gels containing 7 M urea in 1× TBE buffer.

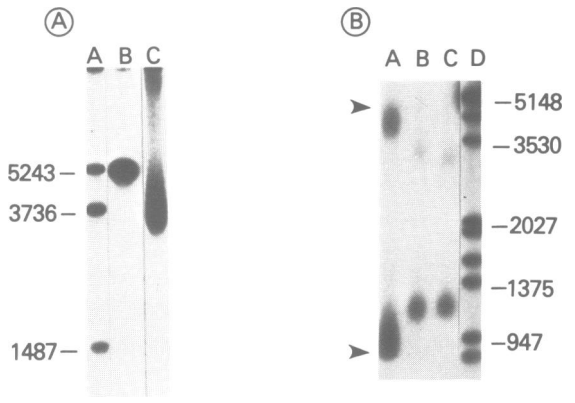

Figure 1. Reconstitution of SV40 DNA fragments. Uniquely 5'-end labeled DNA was mixed with HeLa cell mononucleosomes in 0.70 M NaCl and then the solution was slowly diluted ten-fold. Products of reconstitution were applied to 0.7% agarose gel in 0.5×TBE. **A)** 5188-bp Ban I/Hpa II DNA fragment: lane A, marker DNA; lane B, naked DNA; lane C, nucleosome-reconstituted DNA (nucleosome core particles/DNA ratio = 20). **B)** 4445- and 798-bp Ban I/Taq I DNA fragments (indicated by arrows to the left): lanes A, B and C, reconstituted DNA at nucleosome core particles/DNA ratio of 1, 20 and 50, respectively; lane D, Hind III/Eco RI lambda DNA markers (numbers indicate their size in bp).

Genomic mapping of DNA breaks

Autoradiography films of agarose gels were scanned with a DU-8B Beckman spectrophotometer set at 555 nm, as described previously (21). The densitometer was connected to a computer in order to store, graph and analyze the data. Regression lines of the logarithm of the fragment size (in base pair) versus the migration distance of each fragment from reference line were determined for the DNA markers. Each autoradiography lane was analyzed by using the same reference lane and the size of each DNA fragment was computed. A final correction was made to determine the genomic position of DNA breaks by taking into account the position of the labeled nucleotide relative to the conventional zero position of the SV40 genome (26).

RESULTS

Nucleosome reconstitution of SV40 DNA

Reconstitution was first assessed by an agarose gel band shift assay (Figure 1). In this assay, the mobility of the Ban I/Hpa II nucleosome-reconstituted DNA fragment was consistently accelerated with respect to the corresponding naked DNA fragment (Figure 1A). Similar observations have been reported by Stein (27). This increased electrophoretic mobility was not due to a salt-condensing effect on the nucleoprotein complexes, as it was also observed in low salt buffer agarose gels (10 mM Tris-Cl, pH 8, 1 mM EDTA) (not shown). Nucleosome reconstitution of other DNA fragments showed that the shifting direction was dependent on the fragment length, as a reconstituted 4445-bp fragment was accelerated while a 798-bp fragment was retarded (Figure 1B). Nevertheless, in all experiments, at least 80% of the DNA yielded a homogeneously shifted DNA band.

Nucleosome formation was also assessed by comparing the patterns of micrococcal nuclease cleavage in naked and in nucleosome-reconstituted DNA (Figure 2, A and B). Naked DNA was cleaved by micrococcal nuclease at many sites, and the genomic distribution of the sites was similar in both fragments. Nucleosome-reconstituted DNA yielded a different pattern of micrococcal nuclease cleavage. Cleavage was reduced at many

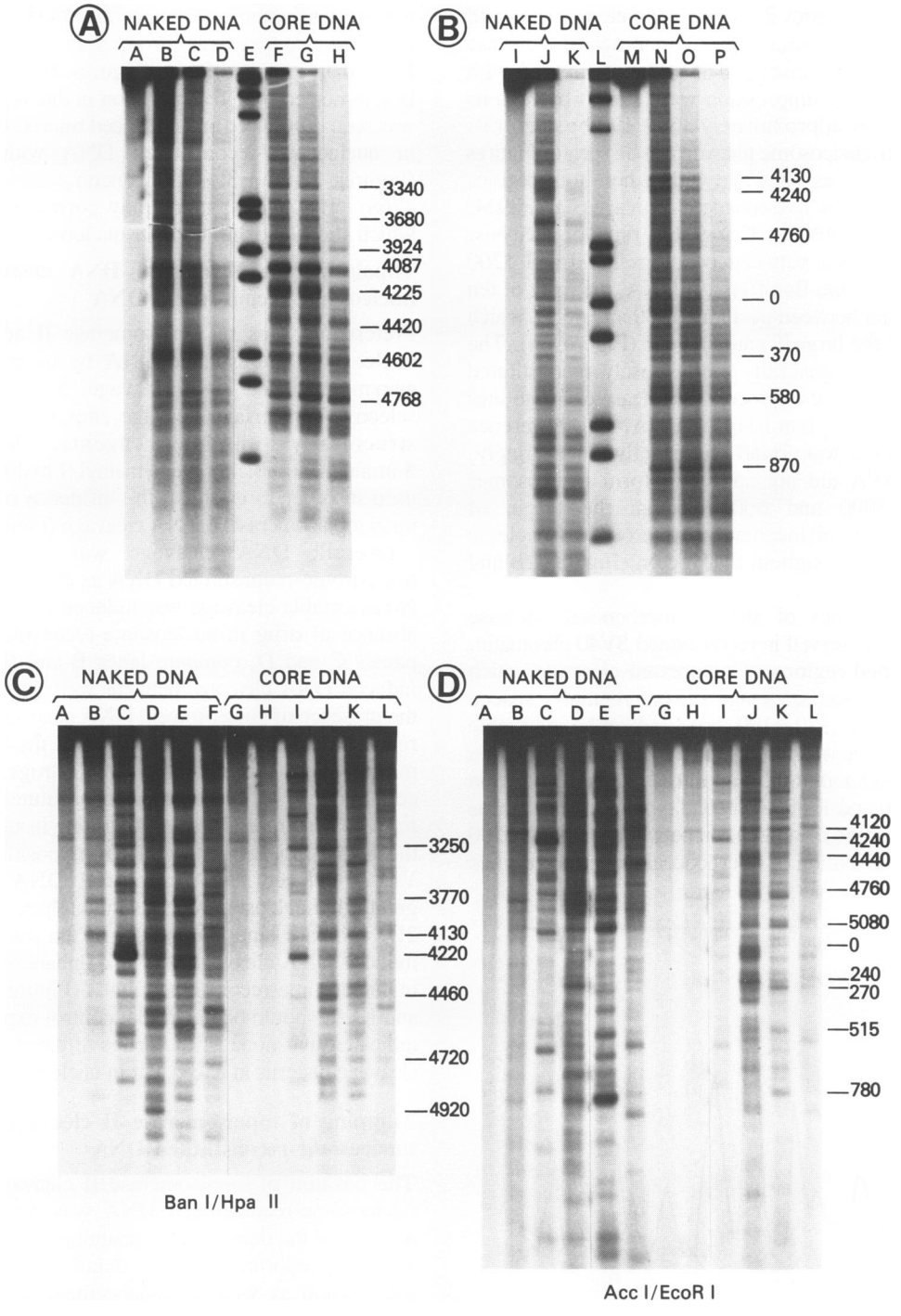

Figure 2. Patterns of micrococcal nuclease (panels A and B) and topoisomerase II cleavage (panels C and D) in naked and nucleosome-reconstituted SV40 DNA fragments. **A)** Micrococcal nuclease cleavage in Ban I/Hpa II DNA: lane A, control DNA; lanes B–D, naked DNA treated with 0.1 mU of micrococcal nuclease for 0.5, 1 and 2 min, respectively; lane E, Hind III/Eco RI lambda DNA markers; lanes F-H, nucleosome-reconstituted DNA treated with 0.05 mU of micrococcal nuclease for 0.5, 1 and 2 min, respectively. **B)** Micrococcal nuclease cleavage in Acc I/Eco RI DNA: lanes I and M, control DNA; lanes J and K, naked DNA treated with 0.1 mU of micrococcal nuclease for 0.5 and 1 min, respectively; lane L, Hind III/Eco RI lambda DNA markers; lanes N–P, nucleosome-reconstituted DNA treated with 0.05 mU of micrococcal nuclease for 0.5, 1 and 2 min, respectively. **C)** Topoisomerase II-induced DNA cleavage in Ban I/Hpa II DNA. **D)** Topoisomerase II-induced DNA cleavage in Acc I/Eco RI DNA. Lanes A and G, control DNA; lanes B and H, DNA treated with 60 ng of murine topoisomerase II without drugs for 20 min; lanes C and I, with 2 μM 5-iminodaunorubicin; lanes D and J, with 10 μM VM-26; lanes E and K, with 10 μM *m*-AMSA; lanes F and L, with 1 μM 2-methyl-9-hydroxyellipticinium. DNA fragments were separated by agarose gel electrophoresis. Numbers to the right of each panel mark the position of the cleavage sites in the SV40 genome.

sites corresponding to nucleosomes and selectively enhanced at other sites corresponding to linkers and nucleosome-free DNA segments (5) (Figure 2, A & B).

Localization of nucleosomes in reconstituted SV40 DNA

The positions of reconstituted nucleosomes were determined by computer analysis of densitometer scanning of autoradiograms

such as those shown in Figure 3. A nucleosome was assigned when a region showed suppression of micrococcal nuclease cleavage in nucleosome-reconstituted DNA as compared with naked DNA and when the suppression was flanked by regions of enhanced cleavage by approximately 150−190 bp apart (5). An example of regular nucleosome phasing can be seen in Figures 2A and 3, between positions 3924 and 4602 where four regions of suppression are flanked by five enhanced cleavage peaks (3924, 4087, 4225, 4420, and 4602). Using this type of analysis, nucleosomes were mapped between positions 3000 and 1200 (Figure 4, top panel). In the Ban I/Hpa II DNA, an array of ten nucleosomes was found between positions 2900 and 4660, which encompass exon 2 of the large T antigen gene (Figure 4A). The Acc I/Eco RI DNA was generally more loosely reconstituted (Figure 4B), as the spacing of micrococcal nuclease cleavage sites was not as regular as in the Ban I/Hpa II DNA. This difference remains unexplained but was found consistently. Interestingly, the Acc I/Eco RI DNA did not appear to form nucleosomes between positions 5000 and 600 (through the origin of replication), as a global enhancement of micrococcal nuclease cleavage was observed throughout that region (Figures 2B and 4B).

In summary, three types of altered micrococcal nuclease cleavage patterns were observed in reconstituted SV40 chromatin. The first type contained regions of suppressed cleavage which were separated by enhanced cleavage sites at regularly spaced intervals (approximately 150−190 bp), indicative of stable nucleosome phasing. This was the case in the early coding region between positions 2900 and 4602, and in the late coding region between positions 600 and 1200. A second type of alteration was observed around the origin of replication between positions 5100 and 580. Here, the distribution of micrococcal nuclease cleavage

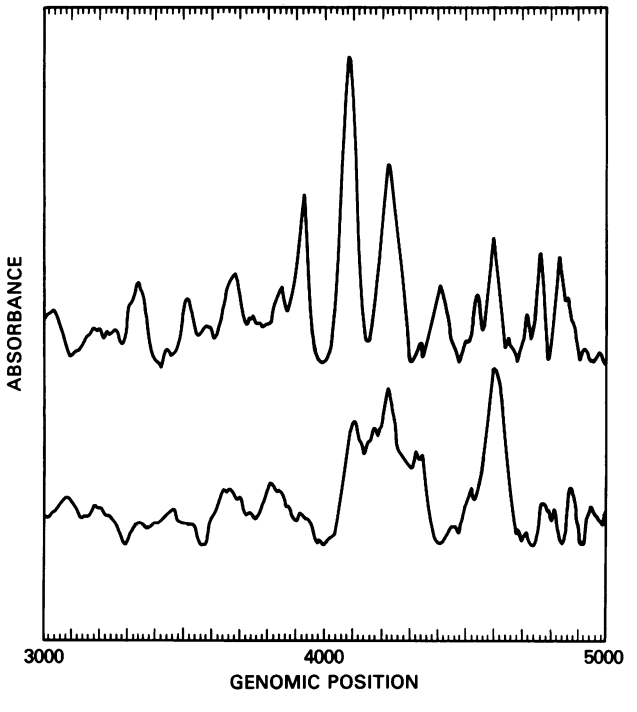

Figure 3. Mapping of micrococcal nuclease cleavage sites in naked and in nucleosome-reconstituted DNA. Lower and upper curves correspond to densitometer scannings of lanes C and F in figure 2A, naked and nucleosome-reconstituted DNA, respectively. The abscissa shows genomic position and the ordinate axis shows optical density in arbitrary unit.

was similar in nucleosome-reconstituted and in naked DNA, but cleavage intensity was stronger in nucleosome- reconstituted DNA than in naked DNA (Figures 2B and 4B), indicative of a lack of nucleosome reconstitution in that region. Finally, a pattern was seen of reduced and enhanced micrococcal nuclease cleavage in nucleosome-reconstituted DNA without regular spacing (Figures 2B and 3, region encompassing positions 4602 and 4768). This third pattern may correspond to DNA segments which did not exhibit stable nucleosome positioning.

Topoisomerase II-induced DNA cleavage in naked and nucleosome-reconstituted DNA

Preferential sites of topoisomerase II action were studied in nucleosome-reconstituted DNA by using drugs that stimulate enzyme-mediated DNA cleavage. Since each class of drug is selective for certain cleavage sites in naked DNA (14), four structurally unrelated agents, VM-26, *m*-AMSA, 5-iminodaunorubicin, and 2-methyl-9-hydroxyellipticinium, were used in order to establish the influence of chromatin structure upon topoisomerase II DNA cleavage (Figure 2, panels C and D).

Overall, DNA cleavage was markedly decreased in nucleosome-reconstituted DNA as compared with naked DNA. No detectable cleavage was induced by topoisomerase II in the absence of drug in nucleosome-reconstituted DNA (Figure 2, panels C and D, compare lanes B and H). In addition, drug-induced DNA cleavage patterns were changed considerably by the presence of nucleosomes. DNA cleavage sites in nucleosome-reconstituted DNA were a subset of those in naked DNA and many of them were common to all drugs, suggesting that only certain regions of nucleosome-reconstituted DNA were available for topoisomerase II action. A striking instance was found around the SV40 replication origin (from position 0 to 350), where VM-26-induced topoisomerase II DNA cleavage was much greater in nucleosome-reconstituted than in naked DNA (Figure 2D, compare lanes D and J). In the same region, *m*-AMSA-induced DNA cleavage was also enhanced around position 270 in nucleosome-reconstituted DNA (Figure 2D, compare lanes E and K). It should be noted that control experiments (not shown) indicated that none of the drugs affected micrococcal nuclease cleavage patterns in naked and in nucleosome-reconstituted DNA.

Mapping of topoisomerase II cleavage sites in naked and nucleosome-reconstituted DNA

The position of topoisomerase II cleavage sites in naked and nucleosome-reconstituted DNA was determined by computer analysis of the densitometer scannings. VM-26-induced cleavage sites are reported in more detail since several instances of suppression as well as enhancement of DNA cleavage were observed for this drug (Figure 2). Figure 3 shows a comparison of DNA cleavage sites induced by VM-26 in naked and nucleosome- reconstituted DNA in relation to nucleosome positions. DNA cleavage was consistently suppressed inside nucleosomes, while it was enhanced in linker DNA and in the nucleosome-free region around the replication origin (Figure 3, lanes C and D). Similar results were obtained in the cases of the other three topoisomerase II poisons (not shown). Furthermore, topoisomerase II cleavage was not decreased in naked DNA incubated in the presence of unreconstituted mononucleosomes, indicating that histone octamers limited topoisomerase II cleavage only when bound to the target DNA molecules. These findings indicate that histone-bound DNA is globally much less accessible to topoisomerase II and that histone binding protects DNA sequences from topoisomerase II cleavage

and increases topoisomerase II cleavage in non-nucleosome regions.

DNA sequencing experiments (not shown) demonstrated that VM-26 induced DNA cleavage at the same in nucleosome-reconstituted and in naked DNA, indicating that nucleosomes did not affect the DNA sequence requirements for topoisomerase II cleavage.

Effects of topoisomerase II on nucleosome positions

In order to test whether topoisomerase II was able to affect nucleosome positioning, Ban I/Hpa II SV40 DNA was reconstituted and then treated with 100 ng of topoisomerase II for 15 min before micrococcal nuclease digestion (Figure 5). The distribution of micrococcal nuclease cleavage sites was not affected by topoisomerase II treatments, suggesting that topoisomerase II was unable to modify nucleosome positions in a linear DNA fragment. However, the global intensity of micrococcal nuclease cleavage was decreased (Figure 5). This observation indicates that topoisomerase II can bind non-covalently to the DNA in nucleosome-free regions. This is consistent with the results of Figure 2 which shows that topoisomerase II cleavage without drugs was abolished in nucleosome-reconstituted DNA as compared with naked DNA (Figure 2, panels B and D, lanes B and H).

DISCUSSION

The nucleosome reconstituted SV40 DNA fragments obtained by means of the histone-exchange method (5) yielded three types of DNA regions: 1) regions with fixed nucleosome positioning; 2) regions lacking regular nucleosome phasing; and 3) a region around the replication origin (from position 5100 to 600) with no detectable nucleosomes. Regions with fixed nucleosomes were similar in two DNA fragments and formed in the coding sequences of both the early and the late genes. Independent studies examining different SV40 DNA regions have also demonstrated the formation of phased nucleosomes around the termination region (28) and in the 'late region' from position 1335 to 1945 (27). Our findings of restricted topoisomerase II cleavage sites in nucleosome reconstituted SV40 DNA fragments are consistent with previous observations in the heat shock locus of drosophila cells (17,19), in the c-myc gene of human small cell lung cancer cells (29,30) and in SV40 (18,31), indicating that topoisomerase II-induced DNA cleavage is globally suppressed in cellular chromatin and that only a subset of the *in vitro* sites are cleaved by topoisomerase II *in vivo*. This can be due to enzyme partitioning in the nucleus or to reduced accessibility of the DNA to topoisomerase II. The present study investigates the latter possibility. The preferential occurrence of topoisomerase II cleavage sites in nucleosome-free DNA segments, and the similarity between the distribution patterns of micrococcal nuclease cleavage sites in reconstituted nucleosomes in the presence or absence of topoisomerase II, suggest that nucleosomes could not be disassembled by topoisomerase II and that, in chromatin, topoisomerase II-DNA complexes may be concentrated in DNA linkers and nucleosome-free regions. In

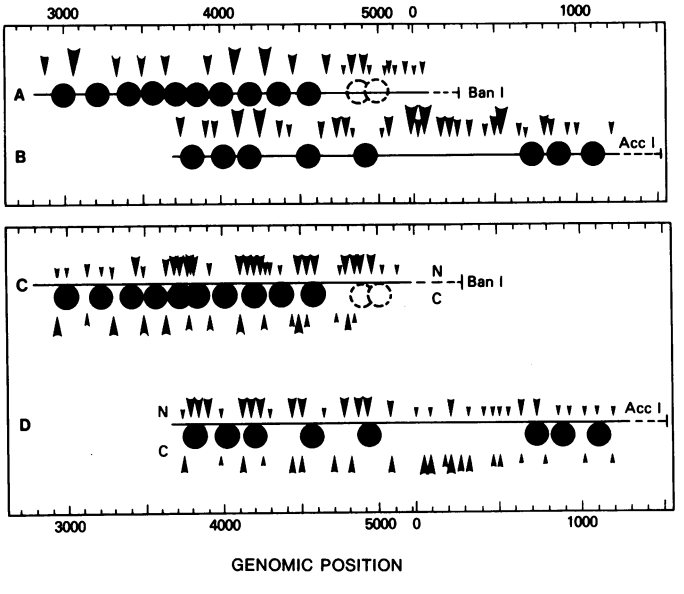

Figure 4. Comparison of micrococcal nuclease (upper panel) and VM-26-induced topoisomerase II cleavage (lower panel) in naked versus nucleosome-reconstituted DNA. Lanes A and B: nucleosome positions in Ban I/Hpa II and Acc I/Eco RI DNA fragments, respectively. Arrows mark sites of micrococcal nuclease cleavage in nucleosome-reconstituted DNA fragments (size indicates the cleavage intensity). Black circles, 150 bp in diameter, represent nucleosomes, and dashed circles represent nucleosomes not uniquely positioned. Lanes C and D: topoisomerase II cleavage sites induced by VM-26 (10 μM) in naked and nucleosome-reconstituted Ban I/Hpa II and Acc I/Eco RI DNA fragments, respectively. Arrows pointing down mark topoisomerase II cleavage sites in naked DNA (N) and arrows pointing up topoisomerase II cleavage sites in core DNA (C) (size indicates the cleavage intensity). Data were obtained by densitometer scanning and computer analysis of autoradiographies (figures 2 and 3 and additional experiments).

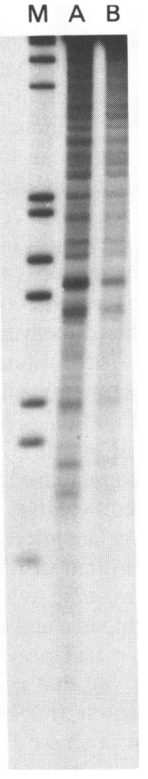

Figure 5. Patterns of micrococcal nuclease cleavage in nucleosome-reconstituted DNA treated with topoisomerase II. Ban I/Hpa II DNA was used in these experiments. Micrococcal nuclease treatments were performed with 0.05 mU of enzyme for 1 min. DNA samples were run into 1% agarose gels. Lane M, Hind III/Eco RI lambda DNA markers; lane A, nucleosome-reconstituted DNA; lane B, nucleosome-reconstituted DNA treated with 100 ng of topoisomerase II for 15 min at 37°C before micrococcal nuclease digestion.

this respect, mammalian topoisomerase II may behave like other DNA binding proteins, including enzymes and regulatory factors whose DNA binding sites are strongly influenced by nucleosomes (32,33). Prior to the present report, the direct interactions of DNA topoisomerases and nucleosomes had only been studied with the prokaryotic enzyme, DNA gyrase in synthetic pBR322 minichromosomes (34). More gyrase was needed in order to reach maximum supercoiling in histone-DNA complexes, and DNA containing 24 nucleosomes or more was completely resistant to the enzyme. The most likely interpretation is that DNA bound around the histone core is inaccessible to DNA topoisomerase II. Thus, our results demonstrate that among nucleoproteins, nucleosomes are important in determining the sites of mammalian topoisomerase II activity.

The modifications of topoisomerase II cleavage patterns in nucleosome-reconstituted DNA appear to be due to changes in enzyme DNA binding affinity rather than alterations in the enzyme recognition sequences since topoisomerase II-induced DNA cleavage sites occurred at similar sites but with different intensity in nucleosome-reconstituted chromatin and naked DNA (not shown). Polyamines also suppress topoisomerase II-induced DNA cleavage at their DNA binding sites and enhance preexisting sites in polyamine-free DNA segments (35). These observations are consistent with the previous findings that eukaryotic DNA topoisomerase II recognizes DNA on the basis of its primary sequence (36–38,20).

The increase in topoisomerase II-mediated DNA cleavage around the replication origin of reconstituted SV40 chromatin is probably related to the lack of nucleosomes in this region (Figures 2 and 4). The fact that this same region corresponds to the nucleosome-free region of a fraction of SV40 minichromosomes *in vivo* (39,40) together with analysis of SV40 mutants (41) indicate that the lack of nucleosomes in the origin region may be due to DNA sequence. It is also interesting to note that that this same region is hypersensitive to psoralen photoadducts and that this effect has been interpreted as evidence for sequence directed structural alterations of the DNA helix around the SV40 replication origin (42). *In vivo* studies of m-AMSA-induced DNA cleavage have shown a major site around nucleotide 270 during late viral infection (18). In the present *in vitro* study, m-AMSA-induced cleavage at site 270 was slightly enhanced in nucleosome-reconstituted DNA. However, in contrast to the *in vivo* experiments, site 270 was clearly not the region of most prominent cutting. Thus, the increased *in vivo* activity of topoisomerase II at site 270 in Yang's system (18) may not be due simply to an increased accessibility of the DNA. It is possible that very specific DNA structural changes occur around nucleotide 270 during late infection and that this specificity is conferred by the nucleoprotein structures existent at that time.

Finally, our data provides some evidence that nuclear matrix, topoisomerase II attachment sites and nucleosomes can occur in the same DNA region. We had previously identified a single nuclear matrix attachment site that maps within exon 2 of the large T-antigen coding region (nucleotides 4071–4377) and coincides with major topoisomerase II cleavage sites (43). It is noteworthy that this same region was hypersensitive to micrococcal nuclease in naked DNA and yielded good nucleosome reconstitution with the expected 150 bp phasing (Figures 2–4). This observation suggests that this region may exhibit particular structural features and that it could either form nuclear matrix attachment sites or yield nucleosomes. The mechanisms governing the balance between these two processes remain to be found. It is possible that only newly replicated SV40 DNA molecules, which lack nucleosome structure, may associate transiently with the nuclear matrix prior to their packaging into minichromosomes. Alternatively, other nucleoproteins may act to disassemble nucleosomes and to render the corresponding DNA regions accessible to topoisomerase II.

ACKNOWLEDGEMENTS

We wish to thank Dr. Brian Imai for providing nucleosome core particles. M. R. was supported by grant GM 29601 from the National Institutes of Health to Dr. E. Morton Bradbury whom we thank for his support during the course of this work. G.C. was supported by awards from Associazione Italiana per la Ricerca sul Cancro and the EORTC-NCI Research Training Program.

REFERENCES

1. Finch, J.T., Lutter, L.C., Rhodes, D., Brown, R.S., and others (1977) *Nature (London)*, **269**, 29–36.
2. Richmond, T.J., Finch, J.T., Rushton, B., Rhodes, D., and Klug, A. (1984) *Nature (London)*, **311**, 532–537.
3. Pederson, D.S., Thoma, F., and Simpson, R.T. (1986) *Annu. Rev. Cell Biol.*, **2**, 117–47.
4. Laskey, R.A., and Earnshaw, W.C. (1980) *Nature (London)*, **286**, 763–767.
5. Drew, H.R., and Travers, A.A. (1985) *J. Mol. Biol.*, **186**, 773–790.
6. Gasser, S.M., and Laemmli, U.K. (1986) *Cell*, **46**, 521–530.
7. Cockerill, P.N., and Garrard, W.T. (1986) *Cell*, **44**, 273–282.
8. Wang, J.C. (1985) *Annu. Rev. Biochem.*, **56**, 665–697.
9. Wang, J.C. (1987) *Biochim. Biophys. Acta*, **909**, 1–9.
10. a rnshaw, W.C., Halligan, B., Cooke, C.A., Heck, M.M., and Liu, L.F. (1985) *J. Cell. Biol.*, **100**, 1706–1715.
11. Earnshaw, W.C., and Heck, M.M. (1985) *J. Cell. Biol.*, **100**, 1716–1725.
12. Gasser, S.M., Laroche, T., Falquet, J., Boy de la Tour, E., and Laemmli, U.K. (1986) *J. Mol. Biol.*, **188**, 613–629.
13. Berrios, M., Osheroff, N., and Fisher, P.A. (1985) *Proc. Natl. Acad. Sci. USA*, **82**, 4142–4146.
14. Tewey, K.M., Rowe, T.C., Yang, L., Halligan, B.D., and Liu, L.F. (1984) *Science (Wash. DC)*, **226**, 466–468.
15. Pommier, Y., and Kohn, K.W. (1989) In Glazer, R.I. (ed.), Developments in cancer chemotherapy. CRC Press Inc., Boca Ranton, pp. 175–195.
16. Liu, L.F. (1989) *Annu. Rev. Biochem.*, **58**, 351–375.
17. Rowe, T.C., Wang, J.C., and Liu, L.F. (1986) *Mol. Cell. Biol.*, **6**, 985–992.
18. Yang, L., Rowe, T.C., Nelson, E.M., and Liu, L.F. (1985) *Cell*, **41**, 127–132.
19. Udvardy, A., Schedl, P., Sander, M., and Hsieh, T-S. (1986) *J. Mol. Biol.*, **191**, 231–246.
20. Fesen, M and Pommier, Y. (1989) *J. Biol. Chem.*, **264**, 11354–11359.
21. Capranico, G., Zunino, F., Kohn, K.W., and Pommier, Y. (1990) *Biochemistry*, **29**, 562–569.
22. Yau, P., Thorne, A.W., Imai, B.S., Matthews, H.R., and Bradbury, E.M. (1982) *Eur. J. Biochem.*, **129**, 281–288.
23. Imai, B.S. (1986) Ph. D. Thesis. University of California, Davis.
24. Imai, B.S., Yau, P., Baldwin, J.P., Ibel, K., May, R.P., and Bradbury, E.M. (1986) *J. Biol. Chem.*, **261**, 8784–8792.
25. Minford, J., Pommier, Y., Filipski, J., Kohn, K.W., Kerrigan, D., Mattern, M., Michaels, S., Schwartz, R., and Zwelling, L.A. (1986) *Biochemistry*, **25**, 9–16.
26. Fiers, W., Contreras, R., Haegema' a Rogiers, R., Van Der Vorde, A., Van Heuverswyn, H., Van Herreweghe, K., Volckaert, G., & Ysebaert, M. (1978) *Nature (London)*, **273**, 113–120.
27. Stein, A. (1987). *J. Biol. Chem.*, **262**, 3872–3879.
28. Poljak, L.G., and Gralla, J.D. (1987) *Biochemistry*, **26**, 295–303.
29. Riou, J-F., Vilarem, M-J., Larsen, C.J., and Riou, G. (1986) *Biochem. Pharmacol.*, **35**, 4409–4413.
30. Riou, J-F., Multon, E., Vilarem, M-J., Larsen, C.J., and Riou, G. (1986) *Biochem. Biophys. Res. Commun.*, **137**, 154–160.
31. Yang, L., Rowe, T.C., and Liu, L.F. (1985) *Cancer Res.*, **45**, 5872–5876.
32. Gottesfeld, J., and Bloomer, L.S. (1982) *Cell*, **28**, 781–791.
33. Emerson, B.M., Lewis, C.D., and Felsenfeld, G. (1985) *Cell*, **41**, 21–30.

34. Garner, M.M., Felsenfeld, G., O'Dea, M.H., and Gellert, M. (1987) *Proc. Natl. Acad. Sci. U.S.A*, **84**, 2620–2623.
35. Pommier, Y., Kerrigan, D., and Kohn, K.W. (1989) *Biochemistry*, **28**, 995–1002.
36. Sander, M., and Hsieh, T.S. (1985) *Nucleic Acids Res.*, **13**, 1057–1072.
37. Spitzner, J.R., and Muller, M.T. (1988) *Nucleic Acids Res.*, **16**, 5533–5556.
38. Fosse, P., Paoletti, C., and Saucier, J-M. (1988) *Biochem. Biophys. Res. Commun.*, **151**, 1233–1240.
39. Saragosti, S., Moyne, G., and Yaniv, M. (1980) *Cell*, **20**, 65–73.
40. Jokobovits, E.B., Bratosin, S., and Aloni, Y. (1980) *Nature (London)*, **285**, 263–265.
41. Jakobovits, E.B., Bratosin, S., and Aloni, Y. (1982) *Virology*, **120**, 340–348.
42. Ostrander, E.A., Karty, R.A., and Hallick, L.M. (1988) Nucleic Acids Res., **16**, 213–227.
43. Pommier, Y., Cockerill, P.N., Kohn, K.W., and Garrard, W.T. (1990) *J. Virol.*, **64**, 419–423.